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Visual Rhythms in Stomatopod Crustaceans Observed in the Pseudopupil

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Abstract. Many aspects of visual function in animals are influenced by the operation of endogenous rhythms. Using techniques of intracellular optical physiology, I investigated visual rhythms in two species of stomatopod crustaceans (mantis shrimps): *Squilla empusa*, a species active throughout the day and night, and *Gonodactylus oerstedii*, which is strictly diurnal. Reflectance from within the deep pseudopupil of the compound eyes and its change upon stimulation with light were monitored in individual animals in constant conditions for up to two weeks. Both species expressed circadian rhythms in visual function. In *S. empusa*, the pupillary response was much stronger during subjective night; little or no response could be elicited during subjective day. In this species, an endogenous rhythm caused pupillary reflectance to increase during subjective day. Rhythms in *G. oerstedii* were of lower amplitude than in *S. empusa* and were more difficult to detect. The differences between these species, together with the results of other comparative research on visual rhythms in arthropods, suggest that circadian, rhythmic processes are involved in optimizing nocturnal eyes for maximum sensitivity and dynamic range.

Introduction

Endogenous rhythms in visual function are common among animals. Diverse rhythmic phenomena associated with vision occur in both vertebrate and invertebrate species. For example, in the vertebrates, events known to be under endogenous control include photoreceptor membrane shedding (LaVail, 1976), retinomotor movements (reviewed in Levinson and Burnside, 1981; Burnside and Nagle, 1983), and synthesis of mRNA coding for opsin (Korenbrot and Fernald, 1989). Invertebrate visual systems also express rhythmicity in membrane shedding (Nässel and Waterman, 1979; Horridge *et al.*, 1981; Williams, 1982) or preparation for shedding (Chamberlain and Barlow, 1984). Visual rhythms apparently unique to invertebrates include cyclic changes in ERG amplitude (*e.g.*, Aréchiga and Wiersma, 1969; Page and Larimer, 1975; Barlow, 1983; Fleissner and Fleissner, 1985), in rates of action potential production (Jacklet, 1969), and, particularly in arthropods, migration of screening pigment in secondary pigment cells (Welsh, 1930; Kleinholz, 1937; Page and Larimer, 1975; see reviews of Stavenga, 1979, and Autrum, 1981).

In many cases, circadian changes in sensitivity are due primarily to variations in the quantum catch by the photoreceptor cells, produced by alterations either in associated structures such as secondary pigment cells or in the amount of photoreceptor membrane per cell. But in some species, the actual photoreceptor cells can undergo rhythmic changes that affect their ability to respond to the capture of a photon by rhodopsin. For example, in *Limulus polyphemus*, circadian events alter electrophysiological properties of individual photoreceptor cells (Kaplan and Barlow, 1980; Barlow *et al.*, 1987; Kass and Renninger, 1988).

The photoreceptor cells of many arthropod species are independently capable of adjusting their sensitivity to light by mobilizing granules of primary pigment, producing a phenomenon known as the pupillary response (Kirschfeld and Franceschini, 1969; see review of Stavenga, 1979). Arthropod pupillary responses may be observed noninvasively by monitoring light reflected from the deep pseudopupil of the compound eye (Stavenga and Kuiper, 1977; Bernard and Stavenga, 1979; Cronin, 1989); as the pigment granules migrate inwards in response to photic stimulation, reflectance rises. Circadian changes in pseudopupillary appearance and level of reflectance have been

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observed in many arthropod compound eyes (Stavenga, 1977; see review of Stavenga, 1979). The rhythms are apparently due to circadian events in secondary pigment cells, under nervous (Page and Larimer, 1975) or neuroendocrine (Smith, 1948; Page and Larimer, 1975; Hernández-Falcón *et al.*, 1987) control. True pupillary responses, however, are caused by translocations of primary pigments, within the actual photoreceptor cells, in direct response to photic stimulation (Stavenga, 1979). Do these responses also express circadian rhythms? If so, the eyes may be optimized for sensitivity and dynamic range at a particular phase of the diel cycle, under rhythmic control.

In earlier work with the squilloid stomatopod crustacean *Squilla empusa*, I found that it was difficult or impossible to elicit any changes in reflection from the deep pseudopupil during the day, whereas nocturnal stimulation produced large, highly repeatable reflectance increases (Cronin, 1989). In contrast, the gonodactyloid stomatopod species *Gonodactylus oerstedii* and *Pseudosquilla ciliata* expressed pupillary responses no matter when they were stimulated. In this report, I describe experiments testing whether there is a rhythmic component to pupillary function in *S. empusa* and *G. oerstedii*. The results suggest that rhythmic events strongly alter the pupillary responses of *S. empusa* and have a weaker influence on those of *G. oerstedii*.

Materials and Methods

Adult animals were used in all experiments. Work with *Squilla empusa* was carried out at the Duke University Marine Laboratory in Beaufort, North Carolina. Animals were collected locally and maintained either in running seawater tables exposed to indirect, natural daylight through windows along two sides of the room (ambient photoperiod experiments) or in small containers containing natural seawater placed in a chamber with a controlled light:dark cycle (reversed photoperiod experiments). Animals were fed fresh oyster and shrimp meat. Experiments with *Gonodactylus oerstedii* took place in Baltimore, using animals collected in the Florida Keys. These animals were kept in aquaria filled with artificial seawater in a 12 h:12 h light:dark cycle, and were fed frozen shrimp.

Reflectance from the deep pseudopupil was monitored using the techniques of intracellular optical physiology, described in detail in Bernard and Stavenga (1979) and Cronin (1989). Dorsal surfaces of animals were attached, using Scutan dental plastic, to a moveable platform which was then submersed in seawater. During each experiment, water in the experimental chamber (which contained about 1200 ml) was changed occasionally, at irregular times. Animals were not fed during an experiment.

Once mounted, each experimental animal was aligned so that the pseudopupil of either the dorsal or ventral half of the eye (see photographs in Cronin, 1986, and Cronin, 1989) was centered within the field of view of an incident-light, photometric microscope. The entire apparatus was housed in a dark box with a black curtain covering the front, and experiments took place in a room that was completely blacked out and isolated from external sources of light. The central region of the pseudopupil under study, which appeared to glow dully when viewed by eye, was isolated using an adjustable field diaphragm. Reflectance from the pseudopupil was monitored as described in Cronin (1989), using light of wavelengths >720 nm (Schott RG720 longpass filter, used with *Squilla*) or >800 nm (Schott RG800 longpass filter, used with *Gonodactylus*); this source of light illuminated the eye continually throughout each experiment, and by itself caused no measurable pupillary response. At 20- or 30-min intervals, a stimulating exposure automatically was provided, produced by passing light from a 150-W Xenon arc through a monochromator (Oriel 7250 with 500-nm blazed grating), counterrotating 10-cm diameter neutral density wedges, and a linear polarizing filter. All stimuli were confined to the ommatidia contributing to the pseudopupil under study, and were at a wavelength of 500 nm (half bandwidth of 10 nm). They were produced by opening a Uniblitz electromagnetic shutter under the control of a microcomputer, and lasted for 5 min (*Squilla empusa*) or 30 s (*Gonodactylus oerstedii*). Measurements of light reflected from the pseudopupil commenced before each exposure and continued until well afterwards, and data were stored on the microcomputer's hard disk for later analysis. The response for each stimulus was defined as the average reflectance during the final 20% of the stimulation's duration, divided by the average reflectance before stimulus onset and following a period in the dark equal to the duration of the stimulus itself (see also Cronin and King, 1989). Responses are plotted as the percentage change in reflectance relative to the average dark levels.

In some cases, the sensitivity of the pupillary response was measured by providing a series of stimulating intensities over a range of 3.5 to 3.8 density units at steps of 0.5 units. Each series was produced under microcomputer control, by rotating the neutral density wedges to a series of preprogrammed settings. The intensity of the stimulating source was measured for each experiment using a calibrated PIN-10DP/SB photodiode (United Detector Technology) placed at the position of the animal's eye.

Results

Earlier work with *Squilla empusa* had revealed that the ability of an animal to express pupillary responses apparently varied with time. Results of an experiment designed to detect rhythms in responsiveness are shown in Figure 1. The animal, a male of body length (rostrum–telson)

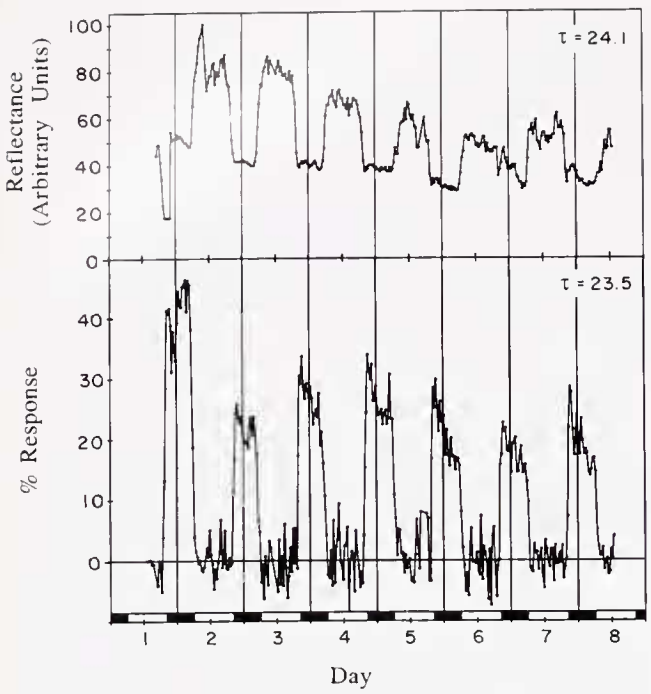

Figure 1. Light reflectance from the deep pseudopupil (Reflectance; top panel) and percentage change in reflectance on stimulation (% Response; bottom panel) in an adult male individual of *Squilla empusa* maintained in constant conditions. Measurements were made at 30-min intervals for a total of 327 intervals, from 7 to 14 July 1987. Stimuli were at 500 nm, at a quantal intensity of 2.9×10^{11} quanta cm^{-2} s^{-1}. The reflectance was measured as described in the text for 1 min before each 5-min stimulation; average values are plotted on a scale normalized to the largest value obtained. Percent response was calculated as described in the text. Vertical lines are drawn at successive midnights. The light and dark bands on the abscissa represent times of natural sunrise and sunset (Eastern Daylight Time). The period (τ) of each rhythm, in h, is given in the top right corner of its panel. Each time series was analyzed using Enright's periodogram technique (Enright, 1965). Periodogram amplitudes were computed at 0.1-h intervals for periods from 10 h to 30 h. The value given on the graph is that of the period in this 20-h range having the greatest amplitude.

85 mm, was placed in constant darkness in the apparatus at midday on the first day and stimulated each 30 min for the next 7 days.

The results of the experiment of Figure 1 are typical of those of most experiments. Rhythmical variations occurred both in the level of the pupillary response and in the reflectance from the pseudopupil in the absence of stimulation. During the subjective day, pseudopupillary reflectance remained high, and little or no measurable reflectance change occurred in response to the light stimulus—the variations that were observed were due to apparently random fluctuations. However, near the time of natural sunset, reflectance from the deep pseudopupil diminished, and large increases in reflectance occurred upon stimulation. Within 1 to 2 h, the reflectance rise during stimulation changed from near zero to greater than 20%. Concurrently, baseline pseudopupillary reflectance decreased by up to 50%. Near the time of subjective dawn, the change in reflectance during stimulation dropped once more to near zero, again over a period of 1 to 2 h, while the baseline reflectance quickly rose to its daytime level. This rhythmical pattern was typical of that expressed in animals maintained under the ambient photoperiod. The period of the rhythm was estimated using Enright's periodogram technique (Enright, 1965); both rhythms had periods near 24 h (see Fig. 1; the difference between the two periods is meaningless with time series of this length).

It is conceivable that the observed rhythms in baseline reflectance and responsiveness were expressions of a single phenomenon; the pupillary mechanism could rhythmically assume its fully light-adapted state during the day, thus increasing reflectance and losing its ability to adapt further to light. Two types of observations argue against this interpretation of the data. First, the two rhythms did not have exactly inverse forms. Baseline reflectance tended to change less abruptly than did the level of response, and on some days its changes were not precisely in phase with the changes in response level. More convincingly, some animals expressed rhythms in responsiveness with little or no circadian change in the baseline reflectance. For example, in the experiment of Figure 2, the baseline re-

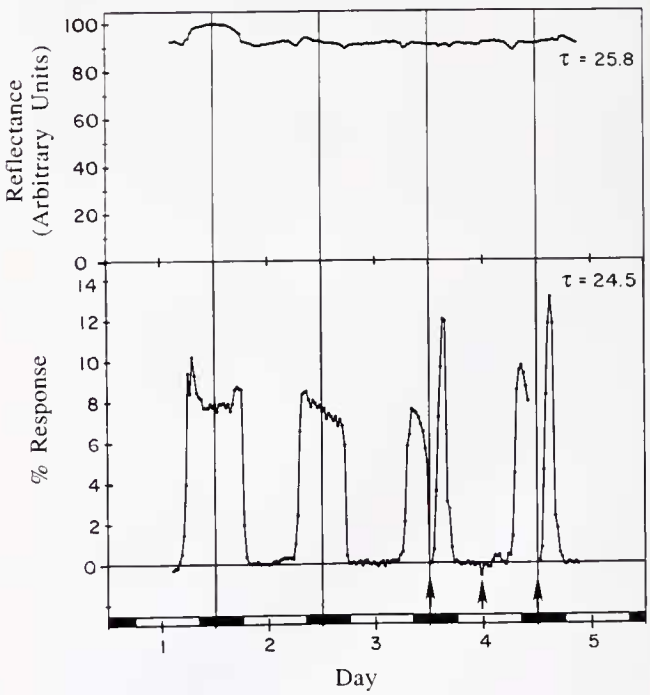

Figure 2. Results obtained from a male *Squilla empusa* maintained in constant conditions from 19 to 23 July 1987. Three series of stimuli of increasing intensities were given, at the times indicated by arrows, for determination of response-*versus*-intensity functions (see text). Except during these series, stimulation was at a quantal intensity of 1.05×10^{11} quanta cm^{-2} s^{-1}. Otherwise as in Figure 1.

flectance varied only slightly over four days. The only changes were a small increase during first dark phase and transient changes on each successive dusk and dawn. These results strongly suggest that the ability of the pupillary mechanism itself to respond to light varies rhythmically. Changes in baseline reflectance could not result from a rhythmical responsiveness to the constant 720-nm light used to monitor reflectance, for such a rhythm should produce increasing pseudopupillary reflectance at night, when responsiveness is greatest. I therefore conclude that although the two rhythms observed in Figure 1 may be intimately linked, they are expressions of separate events within the ommatidia. Once again, periodogram analysis suggests that the rhythms of Figure 2 are circadian. Periodogram analysis is particularly unreliable for time series as short as that of Figure 2, but inspection of the forms of the rhythms clearly confirms that their periods are near 24 h.

The onset of responsiveness at dusk occurs by a smooth transition (Fig. 3). Although successive responses increase in size, the time each takes to reach its plateau phase is similar. It is not primarily the rate of the response that alters, therefore, but the actual amplitude of the reflectance change. The increasing sizes of the responses during the dusk transition mimic the increasing responses that occur with increasing intensity, when the eye is maximally sensitive (Cronin, 1989). The dusk transition, therefore, appears to be a time of rapidly increasing sensitivity of the pupillary mechanism.

The rhythms of responsiveness in these experiments retained phases that appeared to be tightly linked to the external diel cycle, and there remained the possibility that external timing cues were reaching the experimental animals. This possibility was tested by placing an animal in an isolated chamber subjected to a light:dark cycle phased differently from the ambient cycle of sunrise and sunset; lights were switched on at 1800 (Eastern Daylight Time) and off at 0600 each day. Following 12 days of exposure to this "inverted" photoperiod, an animal was placed in constant conditions. Rhythms both of the pupillary response and the baseline reflectance were now observed to be in phase with the imposed cycle. Responsiveness increased, and baseline reflectance decreased, during the entrained dark period between 6 am and 6 pm. To avoid repeatedly stressing the animal by mounting it for study, its visual rhythms were not experimentally defined before imposing the reversed photoperiod. Nevertheless, this was the only case in which maximum responses were ever observed during the astronomical day, so it is reasonable to conclude that the rhythms had been entrained by the exposure to the "inverted" photoperiod. Such results also demonstrate that the rhythms observed in the earlier experiments were not in response to exogenous cues. As before, the periods of the rhythms were very near 24 h (Fig. 4).

Response-*versus*-intensity functions were obtained from the individuals studied in Figures 2 and 4 during both the subjective night and the subjective day. The

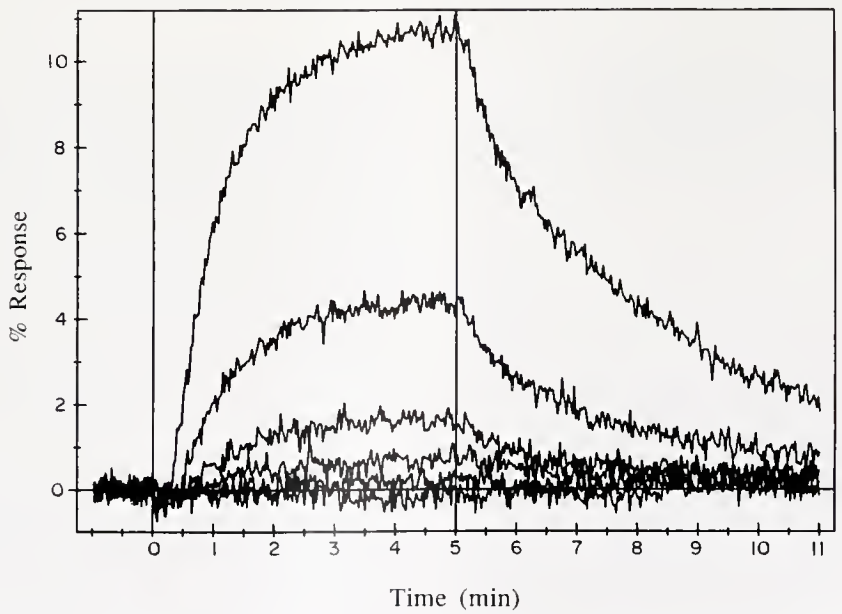

Figure 3. Changes in reflectance from the deep pseudopupil of the animal used in the experiment of Figure 2, during the onset of the nocturnal increase in responsiveness. The 6 traces were obtained at 30-min intervals, beginning at 1535 EDT (lowest trace) and ending at 1805 (highest trace) on 19 July 1987. Percent response is computed relative to the average reflectance in the 1 min prior to stimulation. Vertical lines are drawn at the beginning (0 min) and end (5 min) of the stimulation interval.

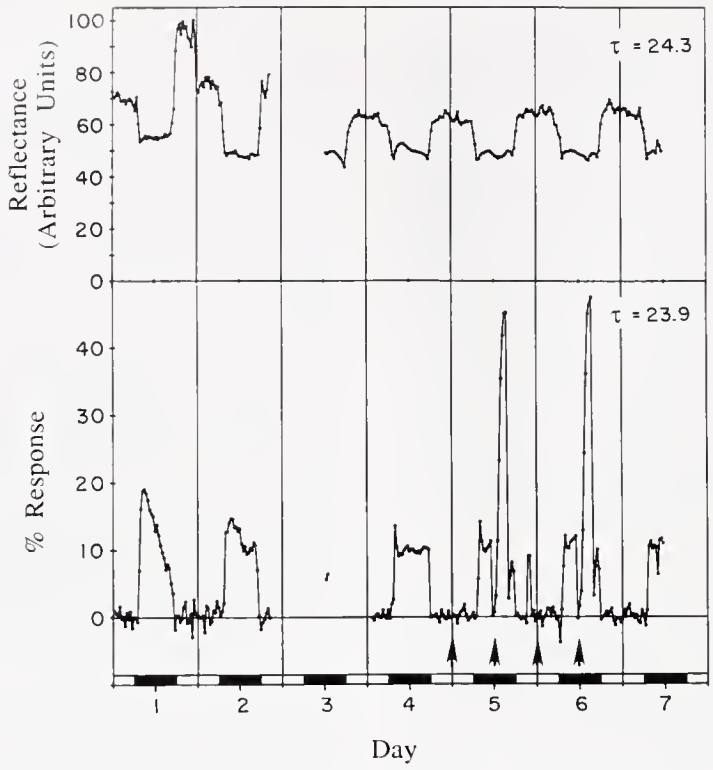

Figure 4. Results obtained from a female *Squilla empusa* maintained in constant conditions from 1 to 11 July 1988. Prior to the experiment, the animal was kept in a controlled light:dark cycle as described in the text; the light and dark bands on the abscissa indicate the times of light and dark of the entraining cycle. Arrows indicate times of the beginnings of series during which stimulation was increased during each half hour. At all other times, stimulation was at a quantal intensity of 1.02×10^{11} quanta cm^{-2} s^{-1}. Gaps in the record indicate times of missing data due to equipment failure; otherwise as in Figure 1.

functions were obtained by stimulating the eye on 8 or 9 successive 30-min intervals with stimuli increasing at steps of 0.5 log units, ultimately providing a maximum quantal intensity of 3.44×10^{12} quanta cm^{-2} s^{-1} (experiment of Fig. 2) or 2.62×10^{13} quanta cm^{-2} s^{-1} (experiment of Fig. 4).

All nighttime series produced response-*versus*-intensity functions of similar shape, with maximal reflectance increases near 13% (Fig. 5A) or 45% (Fig. 5B). In contrast, the daytime response level remained near 0, rising at most to about 3% of the height of the nighttime peak at the maximum stimulation intensity. It appears, in fact, that the maximum pupillary response that can be generated is greatly reduced during the day. To test this conjecture would require stimuli far more intense than what was available in these experiments. The highest intensities to which the animal was exposed during these series were up to 100 times the usual test exposure; somewhat surprisingly, these produced no obvious phase shifts in subsequent cycles of the rhythms (Figs. 2 and 4).

In the earlier work with gonodactyloid stomatopod species (Cronin, 1989; Cronin and King, 1989), no particular diel variation in the pupillary responses was noticed. Nevertheless, it seemed likely that gonodactyloids could possess rhythms similar to those of *Squilla*, but perhaps more subtle in their form. This possibility was tested with *Gonodactylus oerstedii*, using an overall experimental design like that employed with *Squilla empusa*, except that in these cases entrainment was imposed entirely by artificial cycles of light and dark. The pupillary response is much more rapid in *G. oerstedii* than in *S. empusa*, so I used briefer, more frequent stimuli in these experiments. Results of two experiments, representing the range of observed experimental outcomes, are displayed in Figures 6 and 7.

In the experiment of Figure 6, reflectance from the pseudopupil prior to stimulation showed a clear circadian rhythm. Here, the form was rather different from what was obtained using *S. empusa*. Soon after the expected time of lights-out, reflectance from the deep pseudopupil slowly increased, ultimately becoming about 10% greater than during the subjective day. Beginning near midday in the entrainment cycle, this elevated reflectance once more declined. A rhythm in pupillary responsiveness was

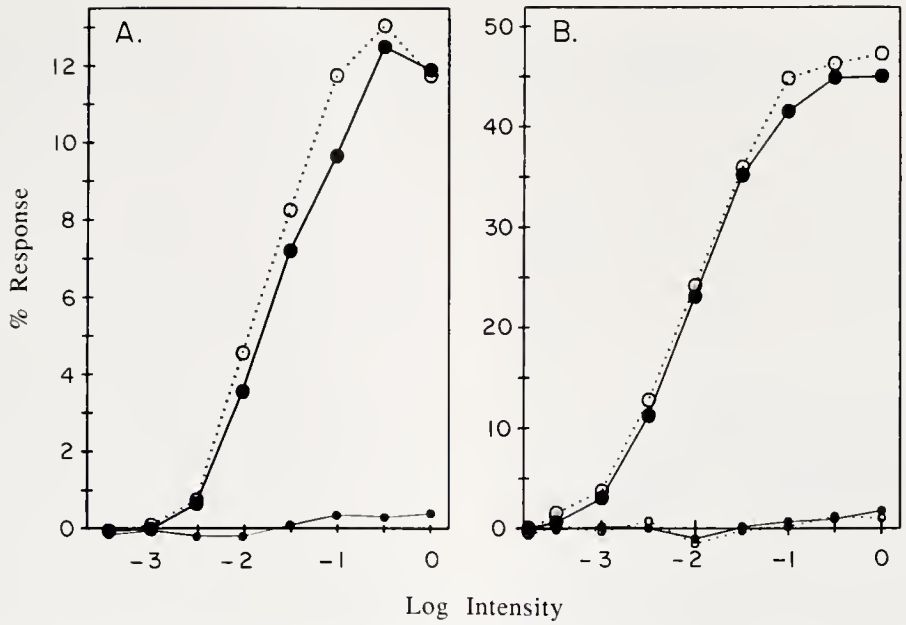

Figure 5. Response-*versus*-intensity functions obtained during the experiments of Figure 2 (A) and Figure 4 (B). Functions were obtained at the times indicated by arrows on those figures; large circles indicate results obtained during subjective night and small circles, during subjective day. Open circles correspond to the first series and closed circles to the second. Photic intensities are relative to 3.44×10^{12} quanta cm^{-2} s^{-1} in panel (A) or 2.62×10^{13} quanta cm^{-2} s^{-1} in panel (B).

more difficult to detect, although on average, responses did appear to be slightly larger at subjective night. Periodogram analysis of the data suggests the presence of circadian cycles in both the baseline reflectance data and the responsiveness data (Fig. 6).

The experiment of Figure 7 revealed the strongest expression of circadian rhythms in responsiveness that I observed in over 20 experiments with *G. oerstedii*. Amplitudes of the pupillary response were about twice as large during subjective day as during subjective night, thus having the opposite form of those expressed by *S. empusa*. The pupillary response was present both during the day and night. In this case, however, there was no evidence of a circadian cycle in pupillary reflectance.

Discussion

As demonstrated here, rhythms with a circadian period can readily be observed through observations of pseudopupils in compound eyes of stomatopod crustaceans. In the squilloid species, *Squilla empusa*, two parallel rhythms of high amplitude are usually observed: a cycle in baseline reflectance from the pseudopupil in the absence of stimulation, and a cycle in the amplitude of the pupillary response to light stimulation. In some experiments, the gonodactyloid *Gonodactylus oerstedii* expressed the baseline reflectance rhythm, although at lower amplitude than *S. empusa*. Rhythms in responsiveness in *G. oerstedii* were more difficult to detect than in *S. empusa*, but they could be observed in some individuals.

The rhythms that were observed were robust. They persisted and maintained their form, frequently with a high amplitude, for a week or more in constant conditions. The rhythms were clearly circadian; their periods were consistently revealed to be near 24 h either by inspection or by periodogram analysis of the data. The rhythm in responsiveness expressed by *S. empusa* was particularly impressive for its rapid rise each subjective evening and its equally rapid fall in the morning.

During the day, the sensitivity of the pupillary response decreased by at least three orders of magnitude. These changes are greater than observed in *Limulus polyphemus*, in which both the electroretinogram (ERG) and single-cell sensitivities decrease by about 1.5 log units (Barlow *et al.*, 1977, 1987). Other arthropods, however, have sensitivity changes between the day and night states about as large as those of *S. empusa*. In the crayfish *Cherax destructor*, dark-adapted single photoreceptor cells are more than two log units more sensitive at night (Bryceson, 1986), while the simple eyes of scorpions rhythmically gain nearly four log units of sensitivity each night (review: Fleissner and Fleissner, 1985). In its natural habitat, *S. empusa* probably lacks a pupillary response during the day. The maximum intensities of stimulation used in producing the response-*versus*-intensity functions of Fig-

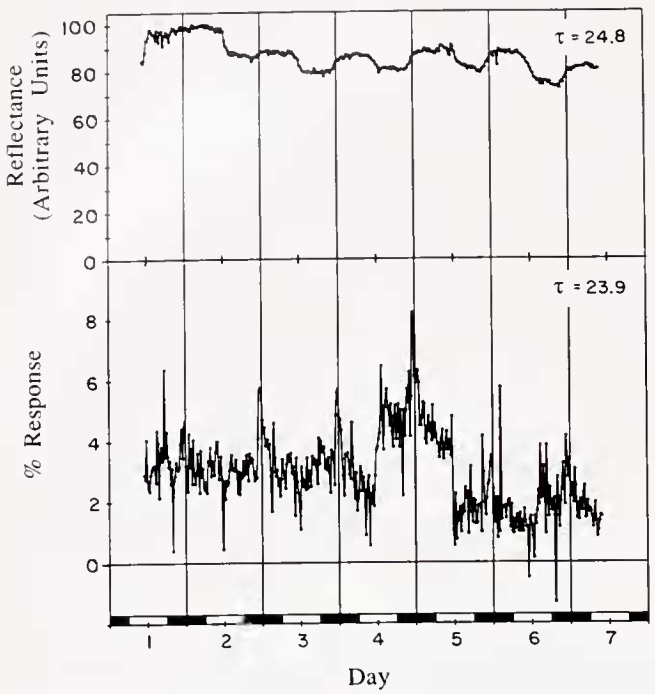

Figure 6. Results obtained from a female *Gonodactylus oerstedii* maintained in constant conditions from 22 to 28 January 1988. The animal was kept in a controlled light:dark cycle, indicated by the light and dark bands on the abscissa, prior to the experiment. Stimulation was at 500 nm, at intervals of 20 min, and at a quantal intensity of 1.57×10^{12} quanta cm^{-2} s^{-1}. Otherwise as in Figure 1.

ure 4, on the order of 10^{13} quanta cm^{-2} s^{-1}, are similar to intensities an animal would experience at a depth of only a few meters in the coastal waters it inhabits (Forward *et al.*, 1988). During the daylight phase of the rhythm, such intensities produced no apparent response.

If these circadian rhythms are to be properly phased to the diel cycle, they must be entrainable by cycles of light and dark. Animals apparently do entrain completely to a novel light:dark cycle within 12 days, as suggested by the results of the experiment of Figure 4. Presumably, photoreceptors for this entrainment are either within the compound eyes or exist elsewhere in the animal. In fact, many invertebrate species entrain their circadian rhythms using extraocular pathways (see review of Bennett, 1979). In crayfish, and probably other decapod crustaceans, photic entrainment of circadian rhythms can be achieved by retinal illumination (Larimer and Smith, 1980), but such entrainment may also involve photoreceptors of the 6th abdominal ganglion (Fuentes-Pardo and Inclán-Rubio, 1987) or other regions of the CNS (Page and Larimer, 1976; Larimer and Smith, 1980). In particular, the work of Page and Larimer (1976) demonstrated that the caudal photoreceptors (in the 6th abdominal ganglion) are not required for entrainment. In contrast to decapod crustaceans, *S. empusa* lacks this caudal photoreceptor (Wilkens and Larimer, 1976), and no other extraretinal photoreceptors have yet been described in stomatopods. If entrainment is mediated solely by the compound eyes, the spatially limited stimuli of these experiments (which were restricted to the ommatidia of the pseudopupil of one part of a single eye) must have been insufficient to induce observable phase changes. Nevertheless, intense stimuli like those used to measure the response-*versus*-intensity functions produce no obvious phase shifts, implying that there may be a role for extraocular photoreception in rhythm entrainment in stomatopods.

What underlying events take place within the compound eyes to bring about the rhythms observed in this study? Changes in the level of baseline reflectance can be effected in several ways (Stavenga, 1979). External to the receptor cells, these include reorganization of optical structures or associated pigment cells, movement of pigment in secondary pigment cells, and masking or unmasking of a tapetum. Within the photoreceptors, changes in reflectance could be caused by alterations in rhabdom size or microvillar organization, or by events within the

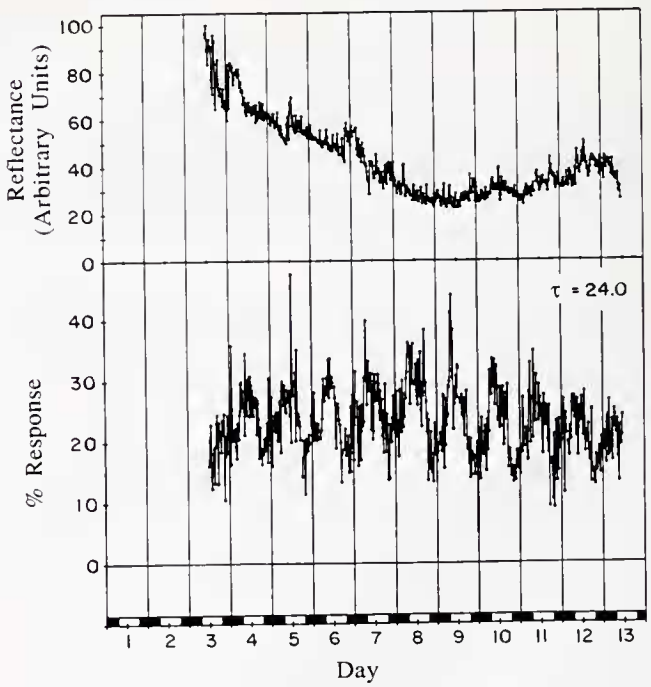

Figure 7. Results obtained from a male *Gonodactylus oerstedii* maintained in constant conditions from 31 January to 12 February 1991. The animal was kept in a controlled light:dark cycle, indicated by the light and dark bands on the abscissa, prior to the experiment. Data from the first two days of the experiment are not plotted, due to repeated animal movement and equipment failure. Stimulation was at 500 nm, at intervals of 20 min, and at a quantal intensity of 1.97×10^{12} quanta cm^{-2} s^{-1}. Periodogram analysis of the data of the upper panel (pupillary reflectance) produced a slowly rising periodogram with a spike peak at 27.1 h; because the analysis produced no clear maximum, the value of τ is not given on the figure. Otherwise as in Figure 1.

cytoplasm of retinular cells, such as rearrangement of the perirhabdomal palisade or movements of pigment granules.

Ommatidial reorganization no doubt accounts for much of the circadian change in the appearance of the pseudopupil of *Limulus polyphemus* (Stavenga, 1979), but more recent work suggests that the retinular cell pigment itself migrates under circadian control (Kier and Chamberlain, 1990). Long-term studies of anatomical changes in stomatopod eyes do not exist. In work with the Mediterranean species *Squilla mantis,* Schiff (1974) stated that during dark adaptation, rhabdoms increase in length and decrease in diameter; simultaneously, the crystalline cone contracts. Schönenberger (1977) also observed the contraction of the crystalline cone during dark adaptation, and noted that the distal pigment cells reorganize at that time. Within the photoreceptor cells, granules of primary pigment line up in neat rows, encircling the rhabdom as it dark adapts. These changes occur during diurnal dark adaptation, following prolonged exposure to daylight, at times when *Squilla empusa* reveals no light-sensitive pupillary responses and no evidence of dark adaptation following stimulation. At present, it is uncertain whether these two species of *Squilla* differ in their ability to respond to light during the day, whether the two sets of studies involve different phenomena, or whether prolonged exposure to light during the day can in fact produce reversible light-adaptation via the pupillary mechanism.

While cyclic restructuring of ommatidia or translocation of secondary pigment could produce rhythmic changes in baseline reflectance, events effecting major alterations in the level of the pupillary response must occur within the actual photoreceptor cells. In apposition eyes like those of stomatopods, the pupillary response is produced by radial translocation of granules of primary pigment residing in the photoreceptors (Stavenga, 1979; King and Cronin, 1989). Reflectance changes may be produced in superposition compound eyes, however, by events in secondary pigment cells (Bernard *et al.,* 1984; Weyrauther, 1986). Compared to the responses in stomatopod eyes, these changes are slow and have considerable inertia—the process continues, or remains saturated, long after stimulation ceases.

Migration of primary pigment is directly under the control of the retinular cell, and, at least in crustaceans, is thought not to be influenced by hormones (Ludolph *et al.,* 1973). In another arthropod, *Limulus polyphemus,* rhythmic neural input does influence the position of primary pigment (Kier and Chamberlain, 1990); similar processes may act in crustacean eyes. The pupillary response requires the presence of calcium ions (Kirschfeld and Vogt, 1980; Frixione and Aréchiga, 1981; Howard, 1984). Since excitation of arthropod photoreceptor cells is also dependent upon intracellular increases in calcium concentration (see review of Fein and Payne, 1989), the absence of the pupillary response during the day could imply that electrophysiological responses of the photoreceptors are also abolished at that time. However, this is unlikely to be the case; Kirschfeld and Vogt (1980) showed that in fly photoreceptors, it is possible to block pigment migration without changing retinal electrical responses. In work with mutant flies, Lo and Pak (1981) also observed that electrophysiological responses could remain in the absence of pigment migration. The processes of pigment translocation and membrane depolarization, while both calcium-dependent, are therefore not completely parallel. The diurnal loss of pupillary responsiveness in *Squilla empusa* could represent another example of the decoupling of these two processes. Experiments are desirable in which the electrical and pupillary responses are monitored simultaneously in this species.

Despite having radically different anatomies, the compound eyes of *S. empusa* and *Limulus polyphemus* are, to some extent, analogous in the functioning of their pupillary responses. Both express rhythmic changes in reflectance from the deep pseudopupil, and both show changes in this reflectance only at night (see Stavenga, 1979). Interestingly, demands on their visual systems throughout the course of each day may also be analogous. Neither species is entirely inactive during the day, but mating of *Limulus,* a behavior involving vision (Barlow *et al.,* 1982), occurs mostly during twilight or night (Barlow *et al.,* 1986). Indeed, nocturnal vision in *Limulus* is extraordinary, enabling the detection of high-contrast targets under starlight (Barlow *et al.,* 1982). Activity cycles of *Squilla empusa* have yet to be examined in the field, but in the laboratory, at least, this species is most active during the night (pers. obs.), and the congener, *Squilla mantis,* is clearly nocturnal (Froglia and Giannini, 1989). The ocular design of *S. empusa* is that of a nocturnal animal (Cronin, 1986). It is plausible that the rhythms in visual function described in this paper are characteristic physiological properties of a nocturnal compound eye. In fact, throughout the arthropods, regardless of eye type, all high-amplitude rhythms in visual physiological function yet described are in nocturnal species. Besides *Limulus,* these include scorpions (simple eyes, rhythm in ERG amplitude and sensitivity: reviewed in Fleissner and Fleissner, 1985), crayfish (superposition compound eye, rhythm in ERG amplitude and sensitivity: Aréchiga and Wiersma, 1969; Page and Larimer, 1975; Bryceson, 1986), and cockroach (apposition compound eye, rhythm in ERG amplitude: Wills *et al.,* 1986).

G. oerstedii, unlike *S. empusa,* is active only from dawn to dusk (Dominguez and Reaka, 1988). At evening twilight, it seals off the entrance to its burrow and remains enclosed all night. Its eyes are optimized for photopic function; indeed, the specialized spectral receptor classes

in its eyes require reasonably bright light to operate at all (Cronin and Marshall, 1989; see also Marshall *et al.*, 1991). Because the eye is used primarily for vision when photon capture is not limiting, strong rhythmic cycles in visual function may be unnecessary.

The expression of high-amplitude circadian visual rhythms in a primarily nocturnal species like *S. empusa*, and their absence in the diurnal *G. oerstedii*, could therefore reveal fundamental differences in function between compound eyes designed for nocturnal (or continuous) *versus* diurnal function. If so, the rhythms observed in this study by monitoring light reflectance from deep pseudopupils are a manifestation of more pervasive underlying alterations that must occur to maintain visual function at day and night. The study of rhythmic cycles of sensory function, and in particular their underlying significance and control, deserves more attention.

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